

**Corrigendum:**

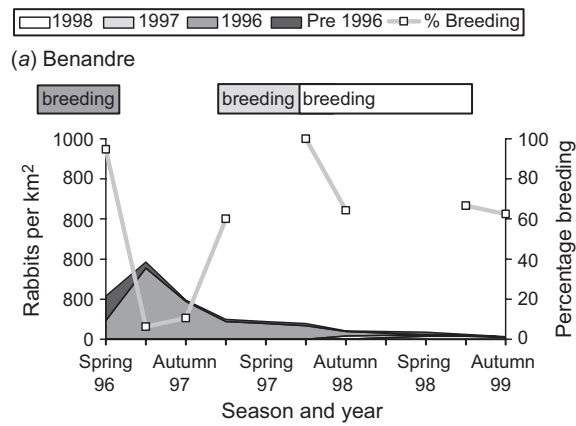
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The impact of rabbit haemorrhagic disease on wild rabbit (*Oryctolagus cuniculus*) populations in Queensland.

G. Story, D. Berman, R. Palmer and J. Scanlan

Fig. 3(a) (p. 189) is incorrect. The correct figure is shown below.

The journal apologises for any inconvenience.



## The impact of rabbit haemorrhagic disease on wild rabbit (*Oryctolagus cuniculus*) populations in Queensland

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**Abstract.** Rabbit haemorrhagic disease virus (RHDV) escaped from quarantine facilities on Wardang Island in September 1995 and spread through South Australia to Queensland by December 1995. To determine the impact of this biological control agent on wild rabbit populations in Queensland, shot sample and spotlight count data were collected at six sites. RHDV spread across Queensland from the south-west to the east at a rate of at least 91 km month<sup>-1</sup> between October 1995 and October 1996. The initial impact on rabbit density appeared highly variable, with an increase of 81% ( $255 \pm 79$  (s.e.) to  $385 \pm 73$  rabbits km<sup>-2</sup>) at one site and a decrease of 83% ( $129 \pm 27$  to  $22 \pm 18$  rabbits km<sup>-2</sup>) at another during the first outbreak. However, after 30 months of RHDV activity, counts were at least 90% below counts conducted before RHDV arrived. Using a population model to account for environmental conditions, the mean suppression of rabbit density caused by rabbit haemorrhagic disease (RHD) was estimated to be 74% (ranging from 43% to 94% between sites). No outbreaks were observed when the density of susceptible rabbits was lower than 12 km<sup>-2</sup>. Where rabbit density remains low for long periods RHDV may not persist. This is perhaps most likely to occur in the isolated populations towards the northern edge of the range of rabbits in Australia. RHDV may have to be reintroduced into these populations. Further south in areas more suitable for rabbits, RHDV is more likely to persist, resulting in a high density of immune rabbits. In such areas conventional control techniques may be more important to enhance the influence of RHD.

### Introduction

Wild European rabbits (*Oryctolagus cuniculus*) were introduced to mainland Australia, in Victoria, in 1859 and spread quickly throughout the southern part of the country, including southern Queensland. Their rate of spread slowed as they moved into less suitable areas (Stodart and Parer 1988) where the winters are too warm and dry. North of the Tropic of Capricorn rabbits are rarely considered a pest (Williams *et al.* 1995). In the southern half of Queensland where suitability is high for rabbits they have caused considerable environmental and economic damage (Williams *et al.* 1995; Berman *et al.* 1998). Robertshaw (1995) estimated that rabbits were responsible for \$A20–65M per year in lost production in the Queensland wool industry alone between 1952 and 1992. Introduction of myxomatosis, European and Spanish rabbit fleas and conventional control have reduced rabbit numbers substantially in the last 50 years (Berman *et al.* 1998). However, rabbits were still a major problem in some southern parts of Queensland prior to the spread of the most recent biological control agent, rabbit haemorrhagic disease virus (RHDV), into wild populations in the State.

The first clinical cases of rabbit haemorrhagic disease (RHD) were reported in China in 1984 (Xu and Chen 1989).

Since then RHDV has spread to become endemic in populations of wild European rabbits in many parts of the world. This includes Australia where, while being evaluated as a potential biological control agent, it escaped from quarantine facilities in October 1995 (Kovaliski 1998). It took two months for RHDV to spread over 700 km from these facilities on Wardang Island in the Spencer Gulf of South Australia to the south-west corner of Queensland (Kovaliski 1998). The Queensland monitoring program was established in mid-1996 to quantify the impacts of RHD on rabbit population dynamics and the subsequent effects of fewer rabbits on vegetation and fauna (Story *et al.* 2000). This paper reports the rate of spread of RHD across Queensland and the impact of RHD on rabbit density. Factors influencing the effectiveness of RHD towards the northern edge of the range of rabbits in Australia are identified and the implications for control activities are discussed.

### Methods

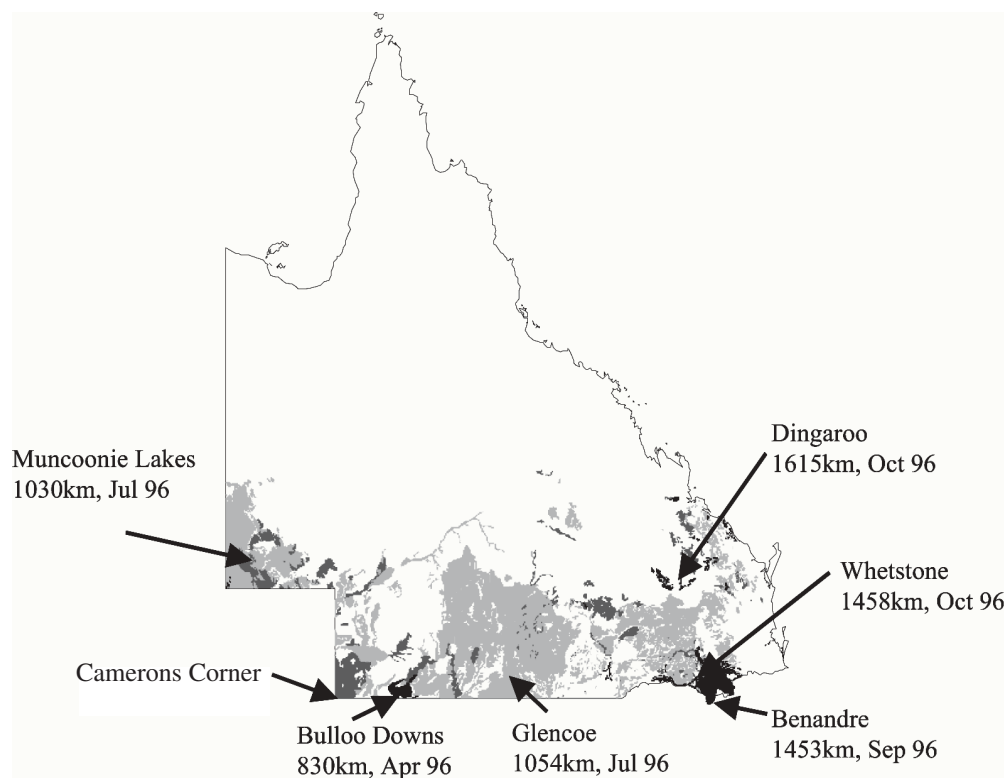
#### Study sites

Six sites were established in southern Queensland as part of the Queensland Rabbit Calicivirus Monitoring and Surveillance program in 1996 (Story *et al.* 2000). Table 1 lists the characteristics of each site including suitability for rabbits based on Berman *et al.* (1998). Figure 1

**Table 1. Characteristics of RHDV-monitoring sites in Queensland**

Suitability for rabbits is based on the density of active warren entrances at 292 Spanish flea release sites expressed as the number of entrances into which fleas were released (Berman *et al.* 1998)

Site	Bioregion	Land use	Suitability for rabbits (release entrances ha <sup>-1</sup> )	Mean annual rainfall (mm) for previous 42 years
Benandre	New England Tableland	Cropping/cattle/sheep	9.5	706
Whetstone	Brigalow Belt	Cattle/cropping/sheep	22.7	610
Dingaroo	Brigalow Belt	Cattle	5.2	619
Glencoe	Channel Country	Sheep/cattle	0.6	330
Bulloo Downs	Channel Country	Cattle	6.0	207
Muncoonie Lakes	Simpson Desert	Cattle	0.1	142



**Fig. 1.** Map of Queensland showing the location of the six study sites and Camerons Corner. Dark shaded areas are the most suitable for rabbits and the lightest the least suitable (from Berman *et al.* 1998). The distance from Wardang Island where RHDV escaped and the approximate date of arrival of RHDV are shown.

shows the location of the sites in relation to areas of different suitability for rabbits. In the most suitable areas, soil is favourable for warren construction and there is a predictable supply of green feed available during the cooler seasons. These factors are conducive to long and productive breeding seasons and rabbit densities are at consistently high levels. For a more detailed site description see Palmer and Story (2000) for Muncoonie Lakes and Story *et al.* (2000) for the other five sites.

Field surveys were conducted monthly at Whetstone, every two months at Muncoonie Lakes and initially every three months at the other four sites. Intervals between field surveys varied because wet weather often prevented access. Autopsy of shot rabbits and spotlight counts were conducted over at least 24 months at all sites other than Glencoe. At Glencoe low rabbit density and the difficulty in completing sampling in the time available made it necessary to cease sampling by shooting after 10 months. Spotlight counts were continued for over 24 months at Glencoe.

#### Shot samples

Rabbits were shot, autopsied and blood and eyeballs collected to determine sex, body condition, age and reproductive and serological status. Approximately 30 rabbits were shot at night during each survey in an area adjacent to the spotlight transect. The location of each rabbit shot was recorded using a GPS and input into ARCVIEW GIS software to determine the proximity to the transect and size of the area sampled. The size of area searched for rabbits to shoot ranged from 8 to 15 km<sup>2</sup> for the six sites. To avoid influencing the population along the transect, rabbits were shot outside the area searched during spotlight counts. The exception to this was Whetstone, where shooting was conducted in the area covered by the spotlight count. This was done for consistency with past data collection at the site since 1993. The greater the distance from the spotlight transect the greater the chance that rabbits would be experiencing different environmental conditions to those along the

transect. There was a need therefore to shoot rabbits as close as possible to the transect but far enough away to avoid influencing the rabbits along the transect. It is unlikely that the small proportion of the population shot (usually less than 1%) significantly influenced the age structure, social structure, density or breeding behaviour of rabbits on the study site.

#### *RHDV antibodies*

Blood samples were taken directly from the heart of freshly shot rabbits. Serum samples were extracted from blood in the field using a centrifuge and were then frozen at about  $-20^{\circ}\text{C}$ . Serum samples were tested by competition enzyme-linked immunosorbent assay (cELISA) for detection of RHDV antibodies (Capucci *et al.* 1991) at the Department of Primary Industries Veterinary Laboratory in Toowoomba. Owing to the large number of samples collected, each sample was assayed at only a single dilution of 1:10. Results from this test are expressed in terms of percentage inhibition in relation to standard positive and negative controls (Cooke 1999). The antibody status of rabbits could then be classified according to 'cut-off' points based on percentage inhibition. The 'standard' cut-off point used was: negative if less than 25% inhibition or positive if equal to or above 25% inhibition. Rabbits that test positive usually have immunity to RHDV. This cut-off point was confirmed as suitable by a challenge experiment conducted on rabbits captured in the wild at Bulloo Downs and in the Stanthorpe region (McPhee *et al.* 2002). The density of rabbits in these classes was determined from total population density estimate and the proportion in shot samples. The proportions of rabbits with or without RHDV antibodies (susceptible or immune) in the shot sample were assumed to mirror the proportion in the population. These proportions were multiplied by the total density to determine the density of susceptible and immune rabbits. Three susceptibility classes were determined: susceptible (<25% inhibition), low positive (25–90% inhibition) and high positive (>90% inhibition).

Low positive percentage inhibitions are potentially due to some factor other than RHDV antibodies since percentage inhibitions of these levels were determined in sera collected prior to the escape of RHDV from Wardang Island. A small proportion (4%) of 278 sera collected from sites in Queensland prior to the escape of RHDV tested above 30% inhibition (Kirkland and Philbey 1999). None of these were over 80% inhibition. In the present study rabbits with sera samples that tested positive with percentage inhibition higher than 90% (i.e. high positives) were considered to have RHDV antibodies. Those positives with lower percentage inhibition may have had either RHDV antibodies or may have tested positive for some other reason, perhaps because of the presence of antibodies to a pre-existing calici-like virus (Cooke *et al.* 2002). The density of rabbits with serum samples above 25% and below 90% was determined as an indication of the maximum possible prevalence of antibodies to this pre-existing calici-like virus. Such prevalence may help explain differences in impact of RHD because antibodies to the suspected pre-existing calici-like virus may protect rabbits against RHDV.

#### *Rabbit age and reproduction*

Eye-lenses were collected from shot rabbits and processed according to the method described by Myers and Gilbert (1968) to obtain an estimate of the date of birth of rabbits. Adult rabbits were classed as those over 20 weeks of age. Female rabbits were considered in breeding condition if pregnant and/or lactating and these data are presented as a percentage of adult females breeding. The usual decline in breeding expected over summer in Queensland (J. Robertshaw, unpublished data) provided a convenient break to distinguish between annual cohorts. Individuals born within the same year were grouped into yearly cohorts. The proportion of a yearly cohort in a sample of shot rabbits was assumed to mirror the proportion in the population. The density of each yearly cohort was

determined as the product of the estimated proportion and total density determined by spotlight count. The age structure of rabbit populations was presented as cohort density to indicate the absolute degree of success of recruitment of individuals from a breeding event and the rate of disappearance of cohorts. This retrospective approach may not give an accurate indication of the relative success of breeding in years or seasons due to differences in reproduction, mortality, immigration and emigration between samples. Observation of very young rabbits, the presence of pregnant and lactating females and estimation of date of birth of shot rabbits combine to determine periods when rabbits were breeding and the time of emergence of new yearly cohorts.

#### *Spotlight counts*

Spotlight counts were used to estimate rabbit density. These were usually undertaken for three consecutive nights along a permanently marked 10-km transect at each site. A trained observer standing in the tray of a vehicle being driven at  $\sim 15\text{ km h}^{-1}$  searched for rabbits with a spotlight (100 W, 500000 candle power). The perpendicular distance from the transect to each rabbit was estimated in 10-m distance classes up to 100 m. Rabbit density was calculated using the Distance V2.0 program (Buckland *et al.* 1993). This program allows for differences in visibility of rabbits under different vegetation conditions. In order to obtain the recommended number of observations (60–80) for determination of density, pooling across surveys within sites was required later in the study, when rabbit numbers were low. The degree of similarity between pooled surveys in the visibility along transects was determined by comparing photographs taken at fixed points.

#### *Arrival and rate of spread*

The time of arrival of RHDV at a site was estimated using data for rabbits that tested positive for RHDV antibodies combined with other indications of the first RHD outbreak detected (described below). Simple linear regression analysis was conducted to determine whether the date of arrival of RHDV depended on the distance of a site from the site of the virus escape, Wardang Island. To determine the rate of spread of RHDV through Queensland it was assumed that the virus was spreading out from Wardang Island. The distance spread from Wardang Island until October 1995 was subtracted from the distance spread until October 1996 to determine the distance spread in that year. Simple linear regression analysis was also conducted to determine whether the proportion of rabbits with RHDV antibodies at a site depended on the distance of a site from Wardang Island. This was done at different times after the escape to determine when RHDV was fully established at the Queensland sites. The assumption was that sites closer to Wardang Island would have a high proportion of rabbits with RHDV antibodies earlier than those further from Wardang Island. Once RHDV was fully established at the Queensland sites no dependence on distance from Wardang Island, and the proportion of rabbits with RHDV antibodies, would be expected.

#### *RHD outbreaks*

The time of occurrence of outbreaks was determined using data for rabbits that had been exposed to RHDV, indicated by the presence of RHDV antibodies (>90% inhibition). The youngest of these from each shot sample was selected. We assumed that RHD occurred sometime between the estimated date of birth and the date that the rabbit was shot. In a population of mostly immune animals the period of exposure was further narrowed since maternal antibodies protect rabbits for at least two months. Usually these rabbits survive through more than one intersurvey period. Those intersurvey periods where rabbits were potentially exposed were further scrutinised for evidence of RHD activity. If there was a significant increase in the proportion of RHDV-immune rabbits and a significant decline in the total rabbit density or no significant increase after a breeding pulse, and no evidence of myxomatosis, an RHD outbreak was considered to have

occurred. A Z-test was used to determine significance at the 5% level (Buckland *et al.* 1993, p. 381). Each intersurvey period was thus classified as having an RHD outbreak or not.

#### *Impact of RHD on rabbit populations*

RHDV was considered to be at a site when an outbreak was detected using the above criteria. The initial impact was considered to be the changes caused by the first outbreak detected. The initial impact of RHD on total density of rabbits, and the proportion of rabbits with antibodies was estimated for each site.

A simulation model was developed to represent the dynamics of a population of rabbits in Queensland (J. C. Scanlan, D. M. Berman and W. E. Grant, unpublished data) using STELLA Ver. 5 software. The model had a weekly time-step with inputs of temperature, forage production, presence of RHD and myxomatosis. Total population of rabbits and those visible by spotlight counting were estimated, with the latter included to enable comparison with field observations.

Rabbit populations were structured by age and by history of exposure to RHDV. Effects of RHD, myxomatosis and natural causes of death were represented and differed depending on exposure history (RHDV), age (myxomatosis, natural causes) and rabbit density (RHDV, myxomatosis). In the model, rabbits born to RHDV-negative mothers can: (1) remain unchallenged by RHDV as they age, (2) become infected by RHDV and survive to become RHDV-positive, or (3) die due to RHD or other causes. Rabbits exposed to RHDV that survive are RHDV-immune for the rest of their life, and die due to non-RHD causes. In the model, rabbits born to RHDV-positive mothers are RHDV-immune during their first two months of life, but then lose their immunity. Natality is affected by warren temperature, forage production, and density of mature rabbits. Forage production was calculated using the 'pasture growth' model, GRASP (McKeon *et al.* 1990). Virulence of RHDV is affected by ambient temperature in the model. The model did not include immigration and emigration of rabbits, and the effects of spatial variability on pasture production and on RHDV activity. It also did not attempt to simulate the time-course of RHD outbreak in a population composed of all susceptible rabbits, i.e. the initial outbreak.

The model was evaluated by simulating population dynamics at Whetstone where data are available before the arrival of RHDV (40 months) as well as after the disease arrived (27 months). The density of rabbits predicted by the model for the duration of the study was not significantly different to density determined by spotlight count.

The model was then used to simulate rabbit densities before and after the arrival of RHDV at the other five monitoring sites. At three of these sites, data were available before and after RHDV arrived. The model was used to simulate rabbit densities both before and after the arrival of RHDV at these sites. These results were compared with spotlight counts and there were no significant differences between observed and simulated populations. Mean simulated densities were then calculated before and after the arrival of RHDV, for each site. At two sites, few or no data were available prior to the arrival of the disease and the modelling approach was the only method that could be used to estimate the impact of RHD on the rabbit population. Also, the density of rabbits that would have been present without the presence of RHDV was simulated with the model.

## **Results**

### *Arrival and spread of RHDV*

RHDV reached Bulloo Downs by April 1996 (Fig. 1) according to the date of birth of a rabbit shot in July 1996 and most likely arrived at Glencoe and Muncoonie Lakes sometime around July 1996. RHDV was officially released

at Whetstone on 14–15 October 1996. Rabbits testing positive to RHDV antibodies were present at Whetstone as early as December 1995. However, on the basis of the rapid increase in the proportion of rabbits with RHDV antibodies after October 1996, it appears that the release was successful or RHDV arrived naturally at about the time of the release. RHDV was released at Benandre on 27–28 October 1996. At that time 46% of rabbits shot there tested positive in the cELISA. Most of these had lower than 90% inhibition and may not have had RHDV antibodies but perhaps possessed antibodies to the suspected calici-like virus (Cooke *et al.* 2002). The fact that one rabbit tested higher than 90% inhibition indicated that RHDV arrived naturally just before September 1996 and was therefore present when the official release occurred. RHDV was released at Dingaroo on 22–23 October 1996, although dead rabbits found one day after the release tested positive to RHDV in the liver, confirming that RHDV had arrived naturally before the release. In a shot sample ( $n = 33$ ) taken in September 1996 there were no rabbits with RHDV antibodies, indicating that RHDV had arrived not long before the release.

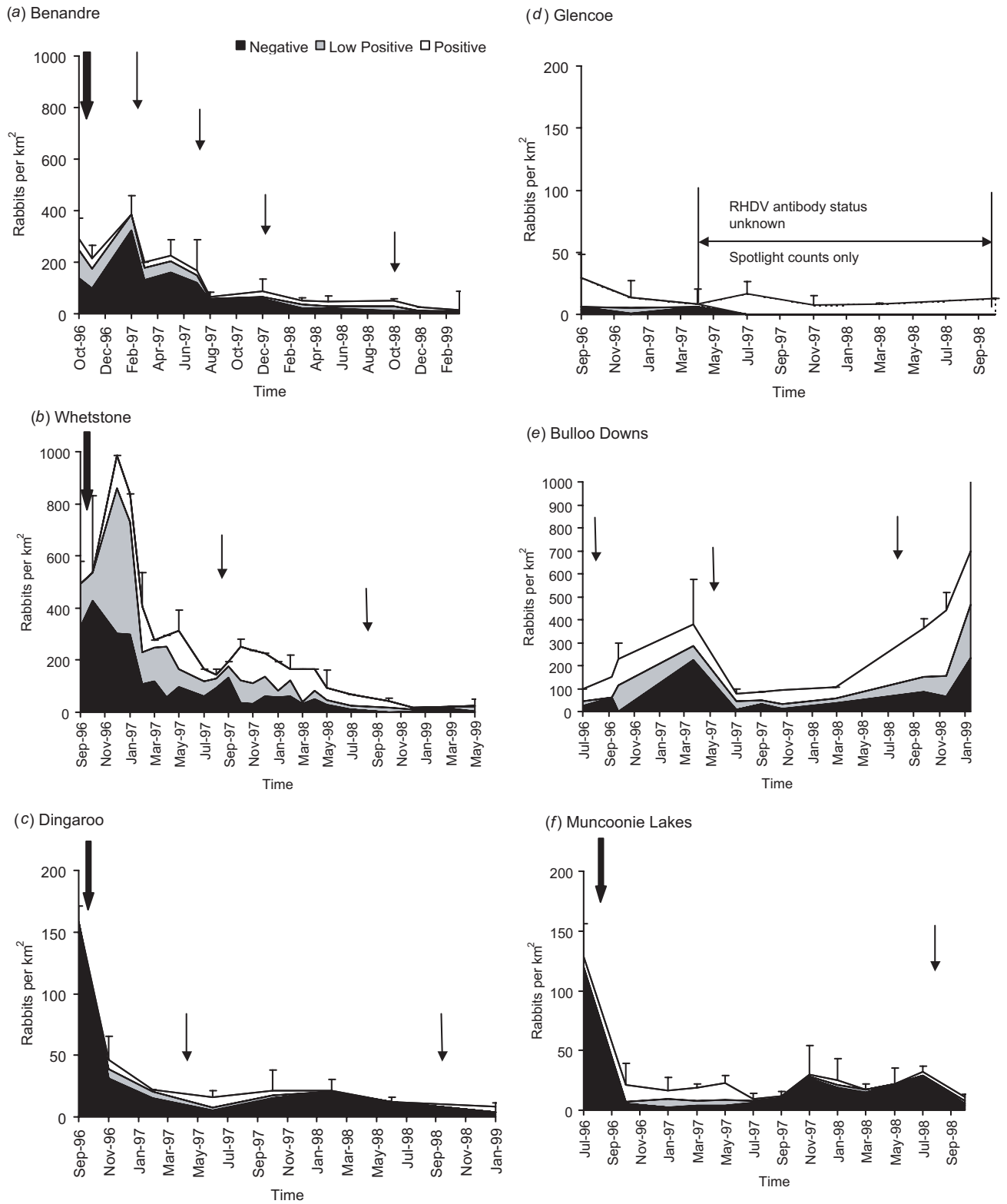
The regression between estimated date of arrival of RHDV at the six sites (Table 1) and distance of the sites from Wardang Island was significant ( $R^2 = 0.90$ ,  $F_{1,4} = 32$ ,  $P = 0.004$ ). The rate of spread of RHDV across Queensland was at least 91 km month<sup>-1</sup> between October 1995 and October 1996. The spread during the cooler part of this period was faster, being at least 129 km month<sup>-1</sup> between April and October 1996.

During the study, sign of RHD was detected 16 times out of a total of 63 periods, all sites combined. On the 47 occasions when sign of RHD was not detected, the level of RHDV activity may have been too low to be detected or else the disease was absent.

### *RHDV activity*

The high proportion of rabbits that tested positive to RHDV antibodies at Bulloo Downs (74%) in July 1996 and Glencoe (79%) in September 1996 show that RHDV was well established there before commencement of sampling. At all other sites, there were low proportions of rabbits with RHDV antibodies (>90% inhibition) when the first samples were collected (Fig. 2), indicating the presence of RHDV before sampling commenced. Fig. 2 shows changes in the proportion of rabbits with or without RHDV antibodies at the six sites. At Muncoonie Lakes the proportion of rabbits with RHDV antibodies increased from 11% in July to 73% in October 1996. Increases also occurred at Whetstone, Dingaroo and Benandre but the time taken to approach similar levels of immunity varied. At Whetstone it took 109 days for the proportion to reach 65%, at Dingaroo 273 days to reach 69%, and at Benandre 395 days to reach 67%.

In October 1996 there was a significant regression between the proportion of susceptible rabbits at a site and the



**Fig. 2.** Rabbit density (mean  $\pm$  s.e.) at the six Queensland RHD sites from July 1996 to May 1999. Density is divided into the proportion of negative (<25% inhibition) (white), low positive (25–90% inhibition) (grey) and high positive (>90% inhibition) (black) antibody levels. Note periods at Glencoe when density was determined but no blood samples were collected. Block arrows indicate the period when the initial outbreak was detected. Line arrows indicate periods of recurring RHD outbreaks.

distance to the site from Wardang Island ( $R^2 = 0.89$ ,  $P < 0.01$ ). This suggests that in October 1996 there was still a relatively high proportion of rabbits that had not been exposed to RHDV in areas most distant from Wardang Island. There was a weaker non-significant relationship in February 1997 ( $R^2 = 0.57$ ,  $P < 0.14$ ) and then no relationship in July 1997 ( $R^2 = 0.02$ ), and December 1997 ( $R^2 = 0.00$ ). Therefore, by July 1997 RHDV was fully established at all six sites.

#### *Rabbit density*

The initial impact on rabbit density is unknown at Bulloo Downs and Glencoe as no counts prior to arrival of RHDV were available. However, there was a high proportion of rabbits with RHDV antibodies in shot samples and the landholder at Glencoe reported noticing a drop in rabbit numbers in July 1996 (J. Burn, personal communication). These indicate that RHD produced a substantial decline in rabbit numbers at this site. At Muncoonie Lakes and Dingaroo, the first outbreak decreased rabbit density by 83% and 70% respectively, and the population remained low for the remainder of the study (Fig. 2). At Benandre there was a 31% decrease in rabbit density from October 1996 to November 1996 after the initial outbreak but rabbit density then increased to be 51% higher than the initial density during December 1996 and January 1997. At Whetstone rabbit density increased 84% during the period when the initial outbreak was detected.

At Muncoonie Lakes the initial decrease of 83% occurred in less than 115 days. The time taken for a decrease of over 80% at Dingaroo was 171 days, at Benandre 517 days and at Whetstone 617 days. The density at the end of the study was 91%, 94%, 95% and 96% (Muncoonie Lakes, Dingaroo, Benandre and Whetstone respectively) below the density determined at the time of arrival of RHDV.

#### *Rabbit population model*

The mean impact of RHD on rabbit populations simulated by the model at the six sites was 74%, with a range of 65–86% (Table 2 – Method 1). An alternative method of estimating impact was to compare the actual spotlight counts after RHDV arrived with the simulated population at those observation dates, had RHDV not been present at the site. This produced a range of 43–94% decrease in population (Table 2 – Method 2). Some differences between the two methods were observed and this was, in part, due to the lower number of observations in Method 2 compared with 24 monthly values in Method 1.

The suitability of the site for rabbits (Table 1; Fig. 1) influenced the reduction in rabbit populations caused by RHD (Table 2), with a significant linear regression using both methods of estimating population decrease (Method 1:  $R^2 = 0.65$ ,  $P = 0.05$ ; Method 2:  $R^2 = 0.82$ ,  $P = 0.01$ ). The Whetstone site exerted high leverage in the regressions.

**Table 2. Rabbit population change using simulated data for the 24 months after RHDV arrived at the site**

In Method 1, the simulated population without RHD was compared with the modelled population with RHD. In Method 2, the observed data were compared with simulated population without RHD for those dates on which observations were made

Site	Method 1	Method 2
Benandre	75%	71%
Whetstone	65%	44%
Dingaroo	77%	87%
Glencoe	76%	94%
Bulloo Downs	70%	63%
Muncoonie Lakes	86%	86%

**Table 3. Suppression of rabbit density due to RHD according to actual population using counts conducted before the arrival of RHD and counts conducted after arrival of RHD**

Site	Actual change in counts from before RHD to after RHD (%)
Benandre	85
Whetstone	67
Dingaroo	87
Glencoe	n.a.
Bulloo Downs	n.a.
Muncoonie Lakes	76

The mean difference between the spotlight count before arrival of RHDV and for the period after RHDV arrived at the four sites where data were available was an 81% reduction in rabbit density (Table 3).

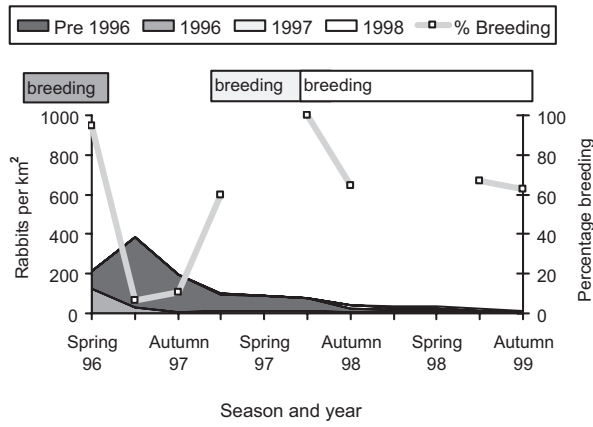
#### *Breeding*

At the time of the first outbreak rabbits were breeding at Whetstone, Benandre and Dingaroo but not at Muncoonie Lakes. The breeding status of rabbits at Bulloo Downs and Glencoe at the time of the first outbreak is unknown.

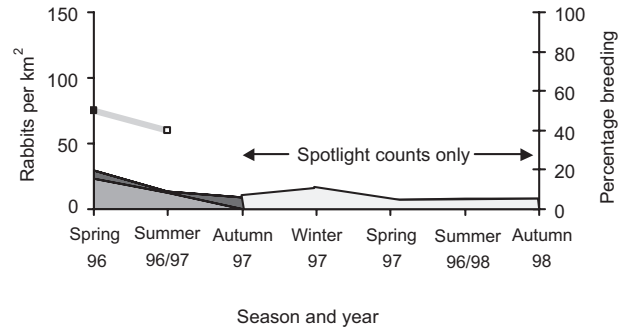
At Muncoonie Lakes and Whetstone, a distinct pattern of high winter breeding rates and low summer breeding rates was recorded. At these two sites there appears to have been a trend towards increased summer breeding rates during the study (Fig. 3).

Breeding was patchier at Bulloo Downs than at any other site, with only three out of eight (38%) seasonal sample periods having high breeding rates (>50% of female adults breeding). All other sites had at least 70% of seasonal sample periods with high breeding rates (Fig. 3). Lack of rainfall appeared to restrict breeding at Bulloo Downs, Benandre and Whetstone in autumn 1997 (Fig. 3; Table 4) when rabbit densities were relatively high. At Dingaroo and Muncoonie Lakes, where rabbit densities were low in autumn 1997, breeding rates were high. Dingaroo had below-average rainfall in 1997 but this did not appear to restrict breeding,

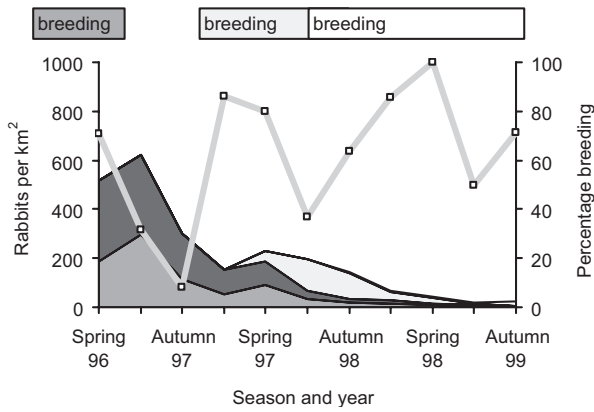
(a) Benandre



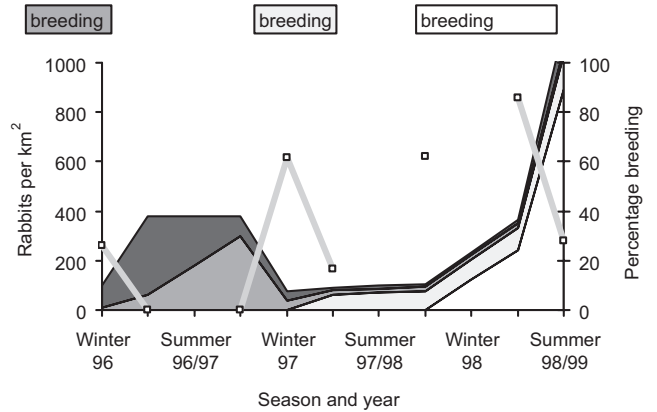
(d) Glencoe



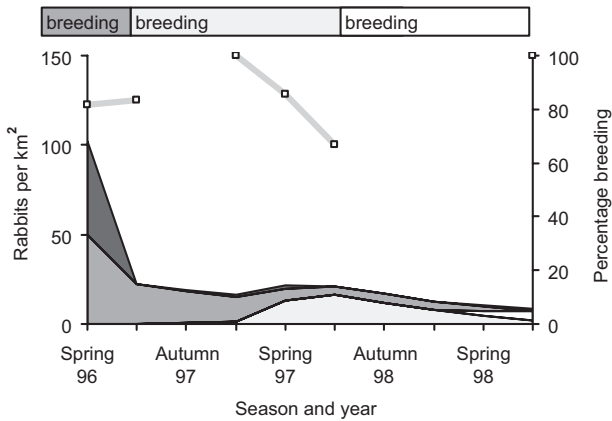
(b) Whetstone



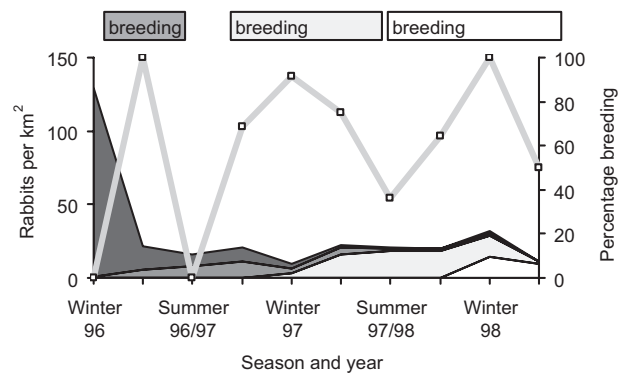
(e) Bulloo Downs



(c) Dingaroo



(f) Muncoonie Lakes



**Fig. 3.** Seasonal breeding presented as the percentage of adult females (>20 weeks) pregnant and/or lactating and rabbit density at five Queensland sites from winter 1996 to autumn 1999. Bars indicate yearly breeding periods. Density (rabbits km<sup>-2</sup>) is divided into the number of rabbits in age cohorts: born prior to 1996 (pre-1996), in 1996, 1997 and 1998.



**Table 4.** Annual rainfall (mm) for each site during the study period

Site	1996	1997	1998
Benandre	1108	741	910
Whetstone	819	602	1027
Dingaroo	214	187	281
Glencoe	373	367	639
Bulloo Downs	174	281	402
Muncoonie Lakes	133	148	138

with over 80% of adult females breeding in all 1997 sample periods. Breeding appears to have been restricted when there was both high rabbit densities and low rainfall.

Fig. 3 shows the breeding periods giving rise to yearly cohorts and the proportional contribution of each cohort to the total density. Each cohort emerged to dominate the population and then gradually declined over 1–2 years.

#### *Density of susceptible rabbits*

The initial density of susceptible rabbits (no RHDV antibodies) ranged from 113 to 310 rabbits km<sup>-2</sup> at sites where surveys commenced before RHD was detected. At Glencoe and Bulloo Downs, where RHDV was endemic when counts commenced, the density of susceptible rabbits was 4 and 16 rabbits km<sup>-2</sup> respectively. At Bulloo Downs the density of susceptible rabbits was high in the 1996/97 and 1998/99 summers (mean = 73 km<sup>-2</sup>) and low in winter sample periods (mean = 13 km<sup>-2</sup>). Once RHDV had become endemic (over 70% of shot rabbits with antibodies and over 80% reduction in total density) at the other sites the density of susceptible rabbits was not found to be above 25 rabbits km<sup>-2</sup> and averaged below 14 rabbits km<sup>-2</sup>. Also, most susceptible rabbits were younger than 12 months old.

No RHD was detected on the 17 occasions when the density of susceptible rabbits was 12 km<sup>-2</sup> or less, representing a large proportion (37%) of the occasions when no outbreak was detected. The absence of RHD at densities of susceptible rabbits 12 km<sup>-2</sup> or less was not due to chance ( $\chi^2 = 8.6$ , d.f. = 1,  $P = 0.01$ ).

#### **Discussion**

RHDV spread through Queensland more slowly than has been reported for other States (Kovaliski 1998). The initial impact of the disease was variable with rabbit density decreasing in some areas but increasing in others. Sites where initial increases were noted had a high proportion of rabbits breeding and/or young rabbits present. By the end of the study, at all sites with pre-RHD counts there were 90% fewer rabbits. Outbreaks did not occur when the density of susceptible rabbits was below 12 km<sup>-2</sup>. Accounting for the influence of rain-induced pasture growth, RHD appears to have suppressed rabbit populations in Queensland by

43–94%. The overall impact of RHD was least at those sites that were most suitable for rabbits.

#### *Arrival and spread*

RHDV was confirmed to be in Queensland by December 1995, near Camerons Corner (Kovaliski 1998), ~700 km from where it escaped from quarantine in September 1995 (Cooke 1997). By October 1996 the virus was confirmed at Dingaroo, 1615 km from where it had escaped.

RHDV spread across Queensland at a rate of at least 91 km month<sup>-1</sup> between October 1995 and October 1996. This was slower than the rate reported for more southern parts of Australia (294 km month<sup>-1</sup>) during the same period (Kovaliski 1998). The slow rate of spread was perhaps a result of the patchier distribution and lower rabbit densities found in Queensland than in the other States. Other factors reported to decrease the effectiveness of RHD, such as high temperature and high relative humidity (Lugton 1999; Henzell *et al.* 2002), may have reduced the rate of spread in Queensland.

#### *Impact on density*

The change in density of rabbits after the first outbreak varied from an 83% decrease to an 81% increase. Saunders *et al.* (1998) reported similar changes in central-western New South Wales, ranging from a 91% decrease to an 87% increase. Caution must be taken when interpreting these changes. An increase of 83% in density after the arrival of RHDV suggests that the disease had no initial impact. However, in the absence of RHD the population may have increased far more. Thus, RHD could have a substantial effect on a population by preventing or restricting an increase as well as by causing a decrease in density.

Although there was great variation between sites in the initial population change after RHDV arrived, the reduction in numbers by the end of the study was very consistent, ranging from 91% to 96%. This was similar to the 95% reduction reported in South Australia by Mutze *et al.* (1998). The population model was used to assess the impact of RHD by simulating the population that would have been present in the absence of RHDV and comparing this with the observed rabbit numbers. This is more appropriate than comparing populations before and after RHDV arrived at a site. A before–after comparison must assume that environmental conditions and rabbit population dynamics (with the exception of RHDV and its impacts) are the same throughout. The more variable the climate, the more unlikely this is to be a valid assumption. In this study the simulations indicated a 74% suppression of rabbit density due to RHD.

Densities at the end of the study were 11–25 rabbits km<sup>-2</sup> at five of the six sites, well below the post-RHD density reported for South Australia (100 rabbits km<sup>-2</sup>). However, the density at Bulloo Downs at the end of the study was 700 rabbits km<sup>-2</sup> or 38 rabbits per spotlight-kilometre. Although

higher than densities reported for South Australia, 38 rabbits per spotlight-kilometre is far below potential densities for the site in the absence of RHD: counts conducted on Bulloo Downs in the early 1990s were over 1000 rabbits per spotlight-kilometre (K. Strong, personal communication). The model predicted that the rabbit density after RHDV arrived was 63–70% lower than if RHD had not been present at Bulloo Downs.

On the edge of the Simpson Desert, at Muncoonie Lakes, the first outbreak of RHD resulted in a rapid decline in rabbit density, as observed in South Australia (Mutze *et al.* 1998). The density of rabbits declined to very low levels and only one further outbreak was detected in the following 29 months. At the other extreme, in the east at Benandre, there appeared to be very little impact on density as a result of the first outbreak. Total rabbit density and the density of susceptible rabbits took four outbreaks and 17 months to reach levels as low as those reached at Muncoonie Lakes in two months. These results support the findings of other studies that showed a higher impact of RHD in arid Australia (Bowen and Read 1998; Mutze *et al.* 1998; Neave 1999), while in wetter regions the impact on rabbit populations has been generally low (Saunders *et al.* 1998; Cooke 1999; Henzell *et al.* 2002).

Most individual RHD outbreaks in this study were not like the initial outbreak at Muncoonie Lakes and did not cause a marked decline in the population; instead, impact was seen as the population failing to increase after breeding. Cooke (1999) has described a similar response to RHD outbreaks in rabbit populations throughout Australia. The result has been an eventual decline to low densities.

#### *Factors that influence RHD*

##### *Suitability for rabbits*

The impact of RHD at Whetstone was less than for other sites. Whetstone was the most suitable of the six sites for rabbits (Table 1). There was a significant regression between impact of RHD and suitability of a site for rabbits, suggesting that rabbit populations remain higher if they can produce more offspring. Other factors may restrict RHD activity in areas that are highly suitable for rabbits. Such factors as a non-virulent calici-like virus (Cooke *et al.* 2002) or heightened resistance to disease may correlate with the suitability of a site to rabbits and may have combined with high breeding rates to reduce the impact of RHD.

##### *Quality of season*

The high density at Bulloo Downs relative to other Queensland sites may be due to the good growing conditions for pasture and the resulting high reproductive rates during 1998. During that year, rainfall was twice the annual average and rabbit numbers increased rapidly. Cooke (1999) considers that high productivity in well watered areas may

act as a factor offsetting mortality due to RHD. With a return to normal rainfall and reduced breeding, rabbit density may decline to levels similar to those recorded at the other five sites.

##### *Rabbit movement*

Another possibility for the relatively high rabbit densities at Bulloo Downs is that the large, continuous area of habitat suitable for rabbits provides a substantial breeding base, with potential for reinvasion after a localised RHD outbreak. Also, rabbit counts may be inflated in summer because of seasonal movements of rabbits. At Bulloo Downs, the density increase corresponded with the end of the breeding season and the pasture dry off (Story *et al.* 2000). Rabbit movements can occur under such conditions (Parer 1982; Twigg *et al.* 1998) as rabbits seek water and pasture (Parer 1982). The Bulloo Downs site was situated along a permanent watercourse. Further work monitoring the dispersal and recruitment pattern of rabbits at Bulloo Downs is required to understand the influence of such factors at this site.

##### *Density of susceptible rabbits*

RHD outbreaks occurred and were detected only when there were more than 12 susceptible rabbits per square kilometre. This is consistent with Lugton's (1999) finding that the prior presence of RHDV reduced the likelihood of an outbreak. During an outbreak susceptible rabbits become infected and they either die or develop antibodies, thus presumably reducing the density of susceptible rabbits. Emergence of a new cohort should increase the density of susceptible rabbits, making conditions more suitable for an RHD outbreak. Outbreaks coincide with the emergence of annual cohorts at many sites throughout Australia (Cooke 1999). A threshold of 12 susceptible rabbits per square kilometre may represent a density below which RHD has no effect, at least near the northern edge of Australia's rabbit distribution. The density of susceptible rabbits was high at Benandre and Whetstone when RHDV was introduced and yet the initial impact was small. A possible explanation for this is that the test used to determine susceptibility is not detecting all immune rabbits and the density of truly susceptible rabbits is indeed much lower than the tests indicate. Robinson *et al.* (2002) demonstrated that maternal antibodies could protect rabbits even when these antibodies are not detectable by ELISA blood test. Antibody titre can also decline in time, to be undetectable if rabbits are not rechallenged (McPhee *et al.* 2002). The technique used in this study may therefore have over-estimated the density of susceptible rabbits.

In this study, few rabbits survived longer than 12 months without being exposed to RHDV and this is consistent with findings in other parts of Australia (McPhee *et al.* 2002).

The few outbreaks detected at Muncoonie Lakes and the absence of rabbits with antibodies recorded in shot samples suggests that RHDV disappeared from the site between September 1997 and September 1998. The low rainfall, myxomatosis and predation may have kept the population at such a low density that RHDV could not be effectively transmitted from rabbit to rabbit, causing local extinction of the virus. Alternatively, RHDV may have been active at low levels undetectable by the sampling techniques used in this study.

### *Breeding*

The reproductive state of the population may influence RHD success. Young rabbits (<8 weeks old) have innate resistance to RHDV (Morrisse *et al.* 1991; Lenghaus *et al.* 1994), although juvenile rabbits may display extremely high mortality rates in the field during an RHD outbreak (S. Ollerenshaw, R. Palmer and D. Berman, unpublished data). Saunders *et al.* (1998) examined three similar sites in central-western New South Wales and found that at the two sites where rabbits were not breeding, RHD produced a substantial decline, while at the third site the population was breeding and RHD appeared ineffective. A similar situation was observed at most of the Queensland sites, with high mortality at Muncoonie Lakes being associated with a non-breeding population consisting entirely of individuals more than 12 months old. Where a high proportion of females were breeding (pregnant or lactating) and there was a high proportion of juveniles present the initial impact appeared minimal. The situation at Dingaroo is inconsistent with this pattern, with a rapid reduction in rabbit density of at least 70% after the initial outbreak even though there was a high breeding rate and juveniles were present in the population. This difference may be due to the reduced suitability of the site for rabbits because of the warmer dryer winters usual for the more northern areas.

### *Other factors*

High temperature and high relative humidity reduces RHDV activity (Smyth *et al.* 1997; Lugton 1999; Henzell *et al.* 2002). At Bulloo Downs during summer the density of susceptible rabbits increased, suggesting that these factors had reduced RHDV activity. Such dramatic increases did not occur at Muncoonie Lakes under similar conditions of temperature and humidity even though breeding was almost continuous there. This suggests that other factors such as predation and/or myxomatosis restricted rabbit population growth at Muncoonie Lakes during the times that RHDV was inactive. Predators can regulate rabbit numbers at low densities and may have done so at Muncoonie Lakes once RHD had reduced the population. While at Bulloo Downs RHD may not have reduced the rabbit population below the critical density required for predator regulation (Banks 2000).

There is some evidence of a calici-like virus inhibiting the effectiveness of RHDV in high-rainfall areas (>500 mm annually) (Cooke *et al.* 2002). This may explain the minimal reduction in rabbits at Benandre and Whetstone after the first outbreak, where annual rainfall exceeds this threshold. Rabbits with low positive serology were most common at Benandre and Whetstone and the density of these rabbits declined at a steady rate during the study. It is possible that this immunity is caused by the calici-like virus (Cooke *et al.* 2002) and, if so, the gradual decline in the proportion of rabbits with low positive serology indicates that the calici-like virus was out-competed by RHDV.

### *Implications for management*

Where the density of rabbits is held at low levels for long periods RHDV may not persist and in isolated populations it may have to be reintroduced. Introduction of RHDV into populations with a low density of susceptible rabbits is not advisable and other control techniques should be used, such as poisoning. With emergence of the next cohort, the population will be predominantly susceptible, improving conditions for RHDV.

In Queensland RHDV is perhaps best introduced into populations with a high density of susceptible, non-breeding, adult rabbits in spring, winter or autumn. RHDV releases can still be effective in breeding populations containing young rabbits but in such cases the density of susceptible rabbits probably needs to be high. Populations with high densities of susceptible rabbits are likely to be most common towards the northern edge of the range of rabbits in Australia, where isolated populations occur and the natural spread of RHDV is unlikely. In the southern parts of Australia where rabbit distribution is more continuous and natural spread of RHDV most likely, conditions suitable for release of RHDV are expected to be less frequent.

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